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13. ABSTRACT (Maximum 200 words) Stochastic sensing with pore-forming proteins has been established as a means for sensing metal ions by using engineered forms of alpha-hemolysin as the sensor elements. The alpha-hemolysin pore is a heptamer, which has disadvantages for certain manipulations in protein engineering. Therefore a single-chain protein pore was sought. In this work, the <i>Escherichia coli</i> outer membrane protein, OmpG, was shown to be a monomeric, single-chain molecule. The availability of milligram amounts of this porin will be useful for membrane protein engineering studies that will yield stochastic sensing elements more diverse than those previously accessible. Analyte-responsive pores are an inherently powerful technology for the real-time quantification of nanomolar levels of essential or toxic metal ions. Genetically engineered pores can be tailored for sensitivity, selectivity, resistance to fouling and other important characteristics. For many metal ions, the response time is limited only by analyte diffusion.					
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## FINAL REPORT

GRANT #: N00014-97-1-0754

PRINCIPAL INVESTIGATOR: Hagan Bayley, PhD; AASERT student: Sean Conlan

INSTITUTION: The Texas A&M University System Health Science Center

GRANT TITLE: The student was be engaged in research under the ONR grant N00014-93-1-0962: Genetically engineered pores for sensing metal ions.

AWARD PERIOD: 1 June 1997 - 31 May 2000

OBJECTIVE: Stochastic sensing with pore-forming proteins has been established as a means for sensing metal ions by using engineered forms of alpha-hemolysin as the sensor elements. The alpha-hemolysin pore is a heptamer, which has disadvantages for certain manipulations in protein engineering. Therefore a single-chain protein pore was sought.

APPROACH: The *Escherichia coli* outer membrane porins form pores that are formed from single polypeptide chains. Unfortunately, most porins are trimeric, which would add another set of complications were they to be used in stochastic sensing. However, a report of a monomeric, single-chain porin, OmpG, appeared in 1998. The evidence for a monomer was weak and therefore a definitive evaluation of the situation was undertaken.

ACCOMPLISHMENTS: A recombinant form of the porin OmpG, OmpG<sub>m</sub>, lacking the signal sequence, was expressed in *Escherichia coli*. After purification under denaturing conditions, the protein was refolded in the detergent Genapol X-080, where it gained a structure rich in beta sheet as evidenced by a CD spectrum similar to that of the native form. Electrophoretic analysis and limited proteolysis experiments suggested that refolded OmpG<sub>m</sub> exists in at least three forms. Nevertheless, the recombinant protein formed uniform channels in planar bilayers with a conductance of 0.81 nS (1 M NaCl, pH 7.5). Bilayer recordings substantiated the proposal that OmpG is a trimer; voltage-induced closures occurred consistently in a single step and channel block by Gd<sup>3+</sup> lacked the cooperativity seen with the trimeric porin OmpF.

CONCLUSIONS: The availability of milligram amounts of a monomeric porin will be useful for membrane protein engineering studies that will yield more diverse stochastic sensing elements.

SIGNIFICANCE: Analyte-responsive pores are an inherently powerful technology for the real-time quantification of nanomolar levels of essential or toxic metal ions in the ocean, waste water and biological fluids. Genetically engineered pores can be tailored for sensitivity, selectivity, resistance to fouling and other important characteristics. For many metal ions, the response time is limited only by analyte diffusion.

PATENT INFORMATION: Gu, L., Braha, O., and Bayley, H. Stochastic sensing mediated by carrier molecules (November 1998, provisional patent filed; November 1999, full patent filed)

AWARD INFORMATION: none

PUBLICATIONS:

Gu, L., Braha, O., Conlan, S. Cheley, S. and Bayley, H. (1999) Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter, Nature 398, 686-690.

Cheley, S., Braha, O., Lu, X., Conlan, S. and Bayley, H. (1999) A functional protein pore with a "retro" transmembrane domain, Protein Science 8, 1257-1267.

Conlan, S., Zhang, Y., Cheley, S. and Bayley, H. (2000) Biochemical and biophysical characterization of OmpG: a monomeric porin, Biochemistry 39, 11845-11854.